

Course 27254

Ex.5

Exercise 5

Differentiation & Colistin Tolerance in *Pseudomonas aeruginosa* Biofilms

What are biofilms?

Biofilms are sessile bacterial communities.

Bacterial cells living in biofilms are:

- attached to a surface,
- or to each other,
- and embedded in an extracellular matrix

Sessile = describes an animal that is permanently attached to something rather than free-moving, e.g. a barnacle



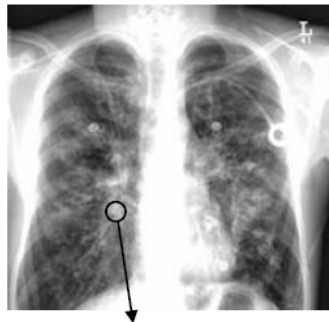
Where do we find biofilms?



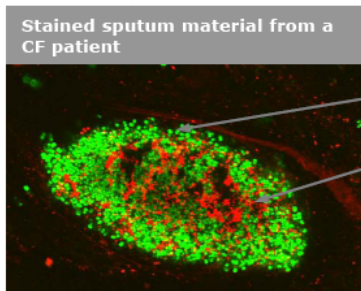
Contact lenses



Medical Devices /
Implants



Lung infections in
Cystic Fibrosis
patients



Stained sputum material from a
CF patient

bacteria (green)

matrix material (red)



Feeding
tubes



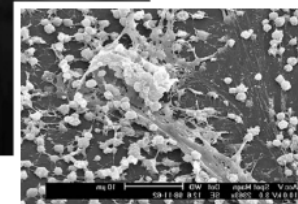
Dr. Norbert Laube with a Stent
University of Bonn, Germany



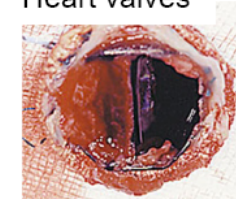
Catheters



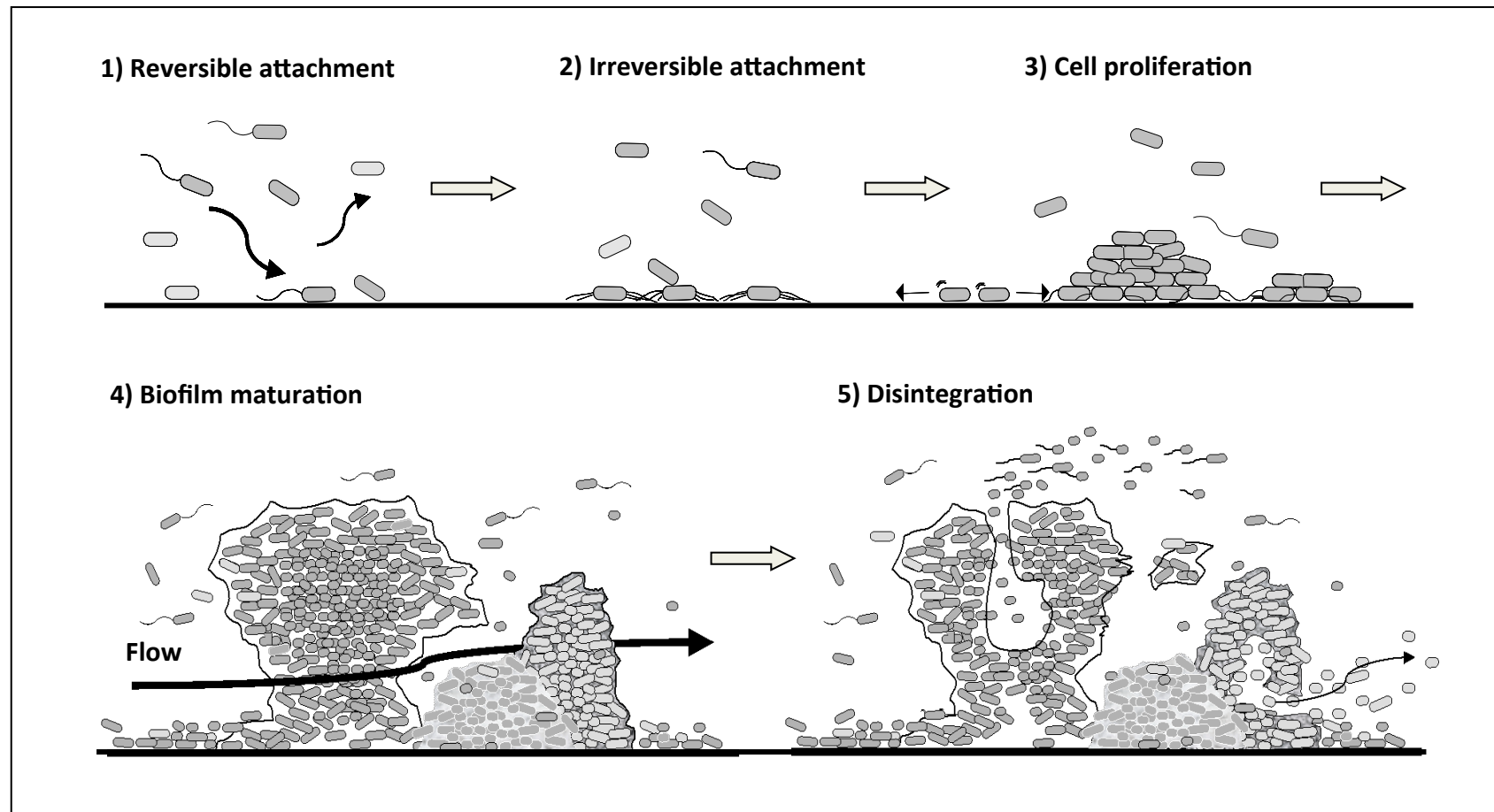
Prosthesis



Heart valves

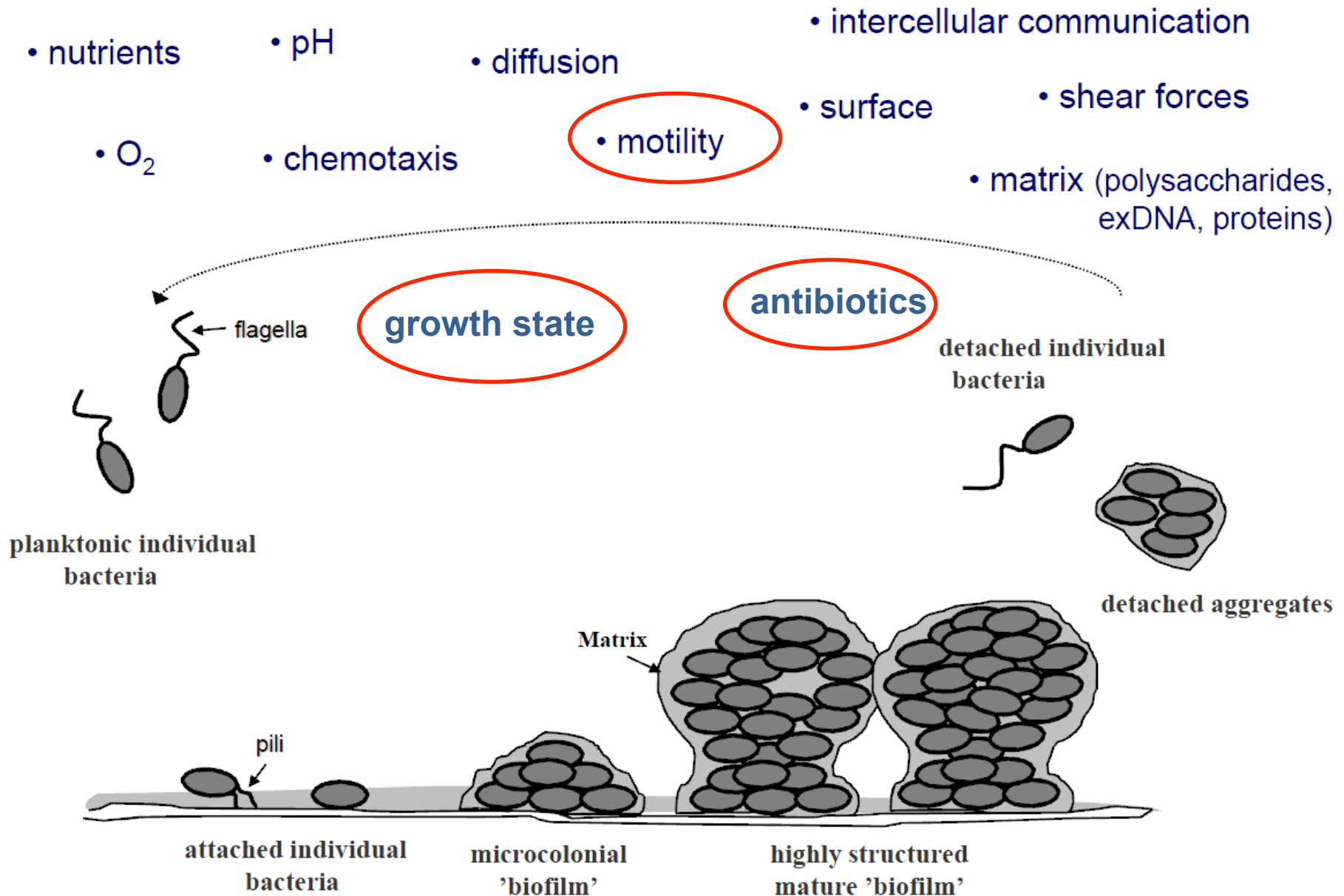


The classical model for biofilm formation.

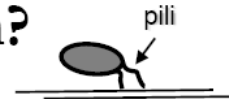


A model of the stages of bacterial biofilm development. At stage 1, the bacterial cells attach reversibly to the surface. Then, at stage 2, the cells attach irreversibly, a step mediated mainly by exopolymeric substances, and the cells lose their flagella-driven motility. At the next stage (3), the first maturation phase is reached, as indicated by early development of biofilm architecture. The second maturation phase is reached at stage 4 with fully mature biofilms, as indicated by the complex biofilm architecture. At the dispersion stage (5), single motile cells (dark cells on the figure) disperse from the microcolonies

Which factors play role in biofilm formation?



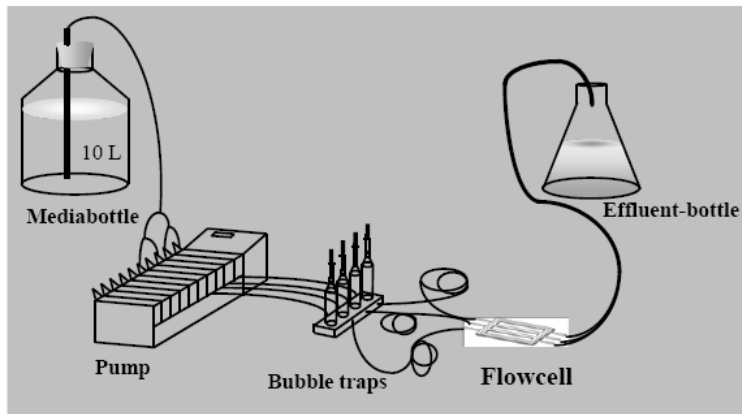
- How does type IV pili-driven motility impact on structural biofilm formation?



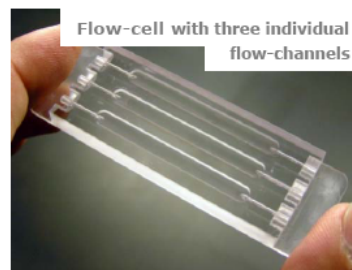
- Which cells/subpopulations in the biofilm are metabolically active, and which are inactive? ↑ ↓
- Which cells/subpopulations in the biofilm are tolerant towards the antimicrobial peptide colistin? 🧫 🧫 🧫

Model System: *Pseudomonas aeruginosa* biofilms grown in flow-cells

Flow-cell setup

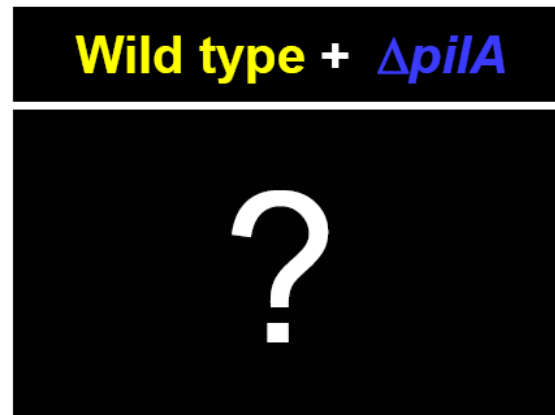
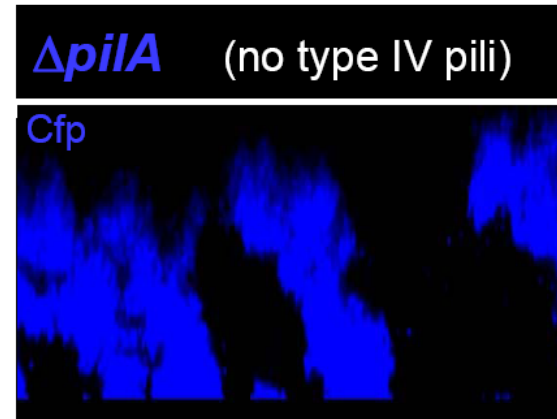
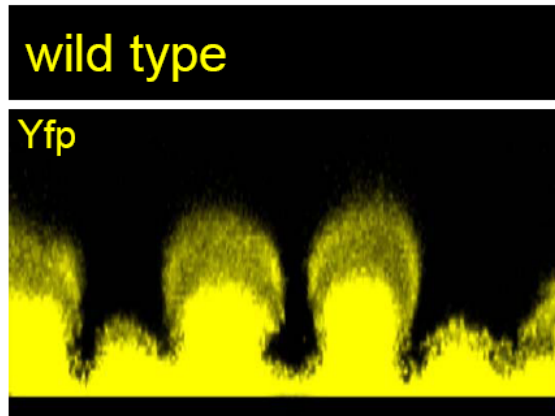


Confocal Laser Scanning Microscopy



Question 1

- How does type IV pili-driven motility impact on structural biofilm formation?

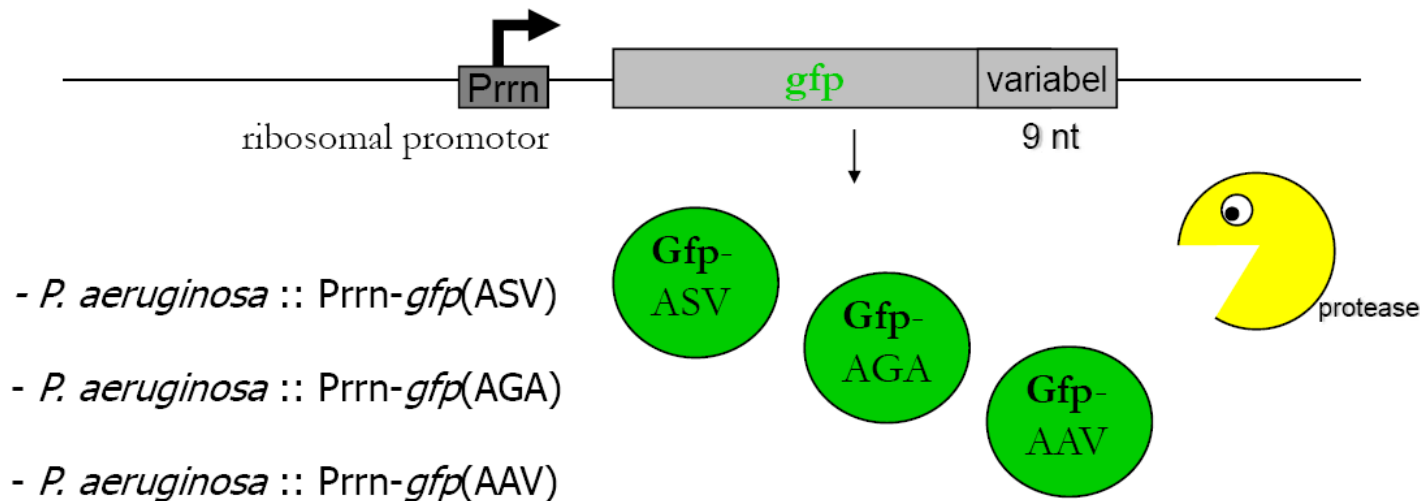


Question 2

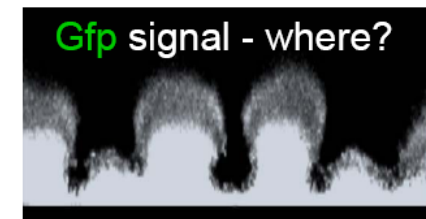
- Which cells/subpopulations in the biofilm are metabolically active, and which are inactive?

P. aeruginosa -

expressing a **fluorescent** growth phase-dependent reporter:



The 3 versions of Gfp have different half lives.



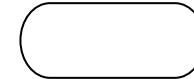
Question 2

- Which cells/subpopulations in the biofilm are metabolically active, and which are inactive?

P. aeruginosa -

expressing a **fluorescent** growth phase-dependent reporter:

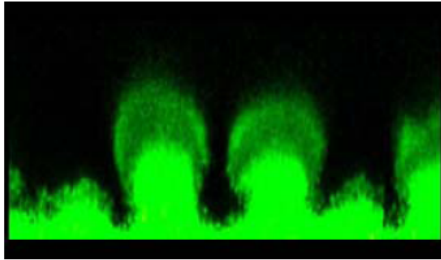
- active cells – **Gfp**-signal
- non-active cells – no Gfp-signal



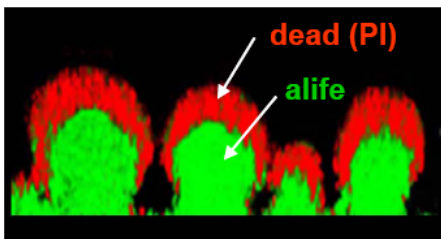
Question 3

- Which cells/subpopulations in the biofilm are tolerant towards Colistin?

wild type



wild type + Ciprofloxacin



wild type + Colistin



Distribution of
dead cells &
alive cells?

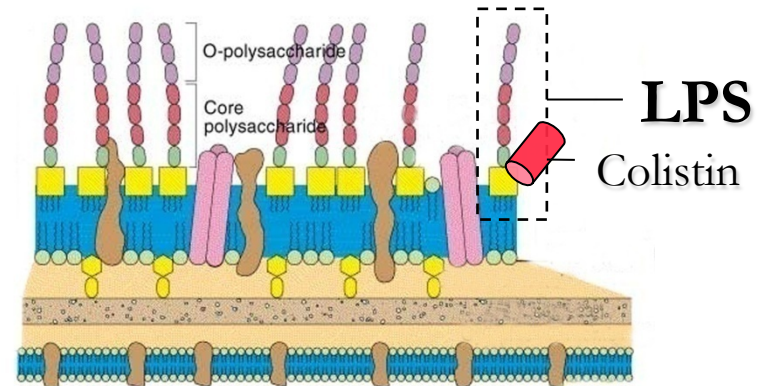
What is Colistin?

- antimicrobial peptide
- synthesised by *Bacillus polymyxa*
- Colistin = Polymyxin E
- has 'detergent-like' properties



Bacillus polymyxa

- Target:
cell membrane of Gram-negative bacteria (LPS - Lipopolysaccharide)
- Mode of action:
 - increase in permeability (pores)
 - leakage of cell content
 - cell death

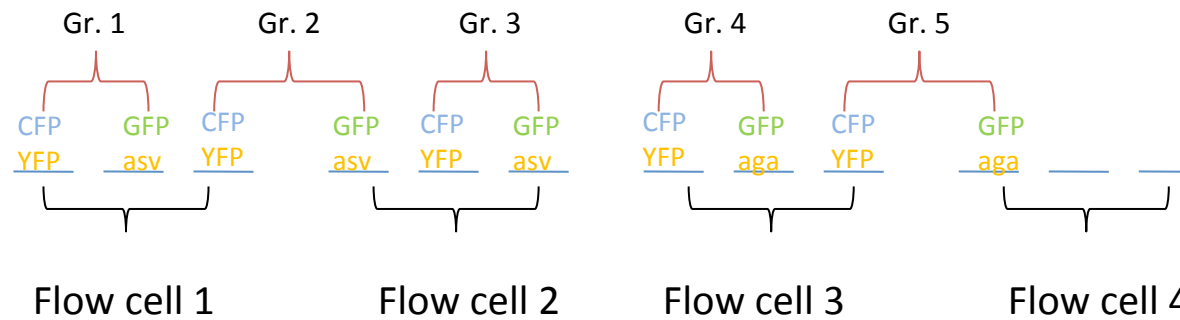


Day 4

Exercise 5 Inoculation overview

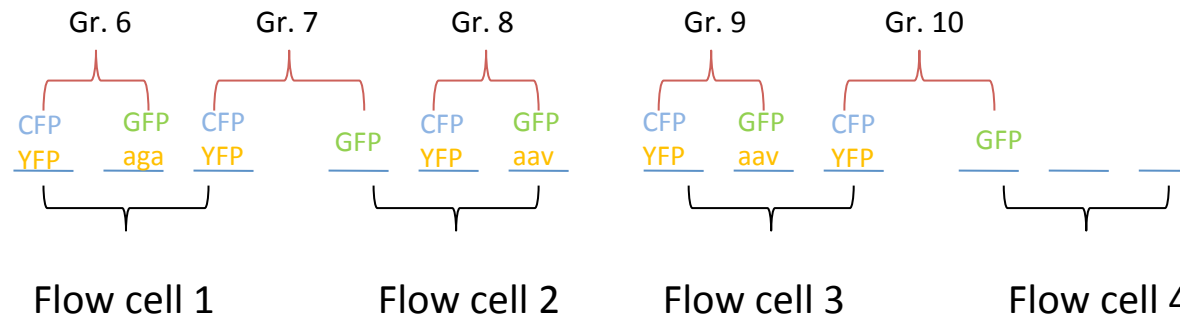
System I

P.aeruginosa pilA-CFP + *P.aeruginosa* WT-YFP
P.aeruginosa -GFP (ASV/AGA/AAV)



System II

P.aeruginosa pilA-CFP + *P.aeruginosa* WT-YFP
P.aeruginosa -GFP (ASV/AGA/AAV)



System III

P.aeruginosa WT-GFP

Colistin treatment – live/dead stain (PI)

